Escape of cultured barramundi (*Lates calcarifer* Bloch) into impoundments of the Ord River system, Western Australia

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Abstract

Mitochondrial DNA sequences were used to compare barramundi found in impounded parts of the Ord River system to their cultured counterparts from a fish farm in one dam (Lake Argyle). Two haplotypes were common to all fish sampled, indicating the high probability that farmed fish are escaping, and could potentially interact with the wild population of the lower Ord River. Although the Lake Argyle barramundi farm currently produces only about 50 tonnes of fish per year, there are plans to expand barramundi production at Lake Argyle by up to 200 times this amount. If that was to occur and no efforts were made to reduce the escape of cultured fish, then sufficient numbers of escaped barramundi may survive to reproduce in the wild fishery. Maintaining the genetic integrity of wild barramundi populations will be best achieved by setting and enforcing standards to minimise escapes from fish farms.

Introduction

Fish are among the world's most intensively cultured organisms. Many species are being propagated for both restocking programs and as founders of novel cultured populations elsewhere, and large numbers of farmed fish are also escaping unintentionally from aquaculture facilities (Bergen *et al.* 1991; Lund *et al.* 1991; Gausen & Moen 1991; Webb *et al.* 1993; Windsor & Hutchinson 1995). A significant frequency and scale of escape of farmed fish has been described by Webb *et al.* (1991) as "inevitable", with numbers of escaped salmonids approaching or exceeding the numbers of naturally produced fish in certain places (Saunders 1991). Thus, the global expansion of fish farming has implications for both cultured and natural fish populations (Fleming & Gross 1993; Hayes *et al.* 1996).

Captive rearing conditions combined with artificial selection, intentional or not, cause fish to diverge from their wild phenotype through environmental and genetic processes (Cross & Challanain 1991; Swain *et al.* 1991). Further, the small numbers of broodstock typically used in fish culture encourage genetic divergence through inbreeding (Tave 1993). There is evidence that fish derived from cultured populations may have both ecological (*e.g.* competition, disease introductions) and genetic (*e.g.* loss of genetic adaptation, genetic homogenization) impacts on wild populations (*e.g.* Rinne & Minckley 1985; Loudenslager *et al.* 1986; Ferguson 1990; Bartley & Gall 1991; Hindar *et al.* 1991; Waples 1991; Wilde & Echelle 1992; Heggberget *et al.* 1993; Fleming *et al.* 1996; Einum & Fleming 1997). There remain, however, few data on exactly how cultured and wild fish interact, and these mostly concern salmonids in the northern hemisphere (Fleming & Gross 1993; Fleming *et al.* 1996; Einum & Fleming 1997).

Barramundi (*Lates calcarifer* Bloch) is a highly fecund and euryhaline fish with a tropical Indo-West Pacific distribution including northern Australia. There are around 30 commercial barramundi farms in Australia, producing approximately 500 tonnes in 1996 (Brown *et al.* 1997). Both the progeny for grow-out operations and the broodstock themselves are typically descendants of those broodstock used to begin the industry in government facilities in Queensland and the Northern Territory some years ago. Therefore, comparatively few broodstock may have founded much of the present barramundi culture industry in Australia. For example, in its few years of operation, the aquaculture facility at Lake Argyle in Western Australia (Fig 1) has reared the progeny of broodstock supplied by the government hatchery in the Northern Territory. No more than four broodstock captured from the coast near Darwin have been used in group larval production for the Lake Argyle farm, with one highly fecund female producing about 75% of all progeny so far supplied (G Schipp, Hatchery Manager, Aquaculture Branch, Department of Primary Industry and Fisheries, Northern Territory, pers comm).

In recent years, there has been an increasing incidence of barramundi caught as a bycatch of the Lake Argyle catfish (*Arius* spp) fishery, and by recreational fishers in Spillway Creek, to which waters from Lake Argyle flow when the lake exceeds the bank-full stage. Spillway Creek joins a natural watercourse (Stonewall Creek) some distance downstream, and then the Lake Kununurra irrigation diversion dam (*i.e.* the Ord River; Fig 1). At least two explanations can account for the presence of

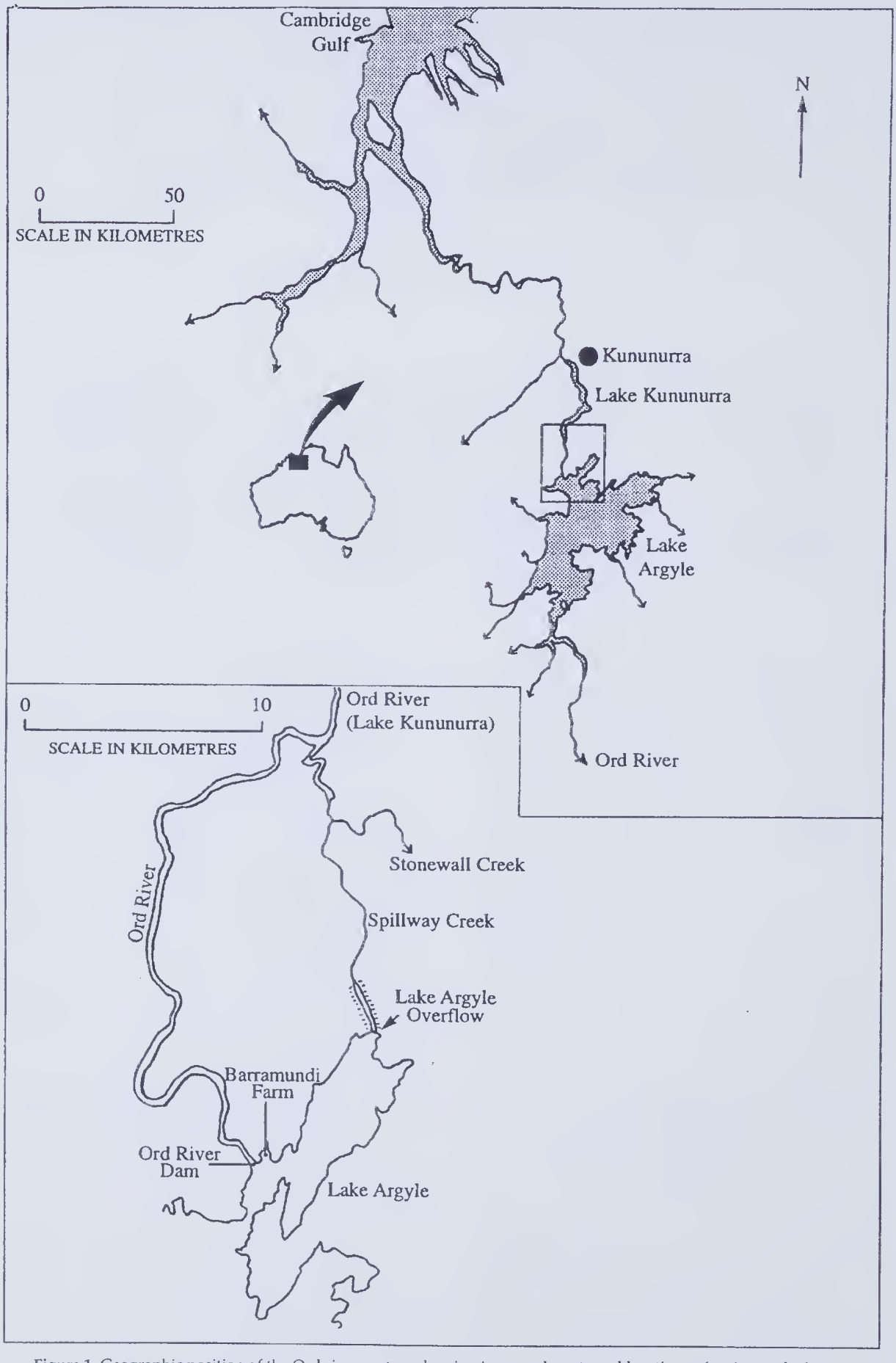

Figure 1. Geographic position of the Ord river system showing impoundments and locations of major study features.

barramundi in these impoundments. Either these fish were captured from the natural barramundi population below the diversion dam wall and have been released into Lake Kununurra, where they have moved upstream as part of their ecological migration, or they are cultured fish that have escaped from farm netpens into Lake Argyle and then into Spillway Creek. From these perspectives, this study aimed to compare the genetic identity of barramundi found in the impounded sections of the Ord River to cultured barramundi from the Lake Argyle fish farm.

Materials and Methods

Barramundi were sampled from the Lake Argyle barramundi farm (7 fish), from the open waters of Lake Argyle (8 fish) and from Spillway Creek (4 fish). For each fish, tissue samples comprising a caudal fin clip and/or muscle tissue (Doupé & Chandler 1998) were placed in specimen vials containing 80% ethanol for 2 hours. Once the alcohol had diffused through the tissue, it was replaced with 70% ethanol for sample storage. Samples were then transported to Perth and processed at the laboratories of the Centre for Human Genetics at Edith Cowan University, using the methods of Doupé & Chandler (1998) and Doupé *et al.* (1999).

Briefly, DNA was extracted from the tissue samples and primers described by Chenoweth *et al.* (1998) were used to amplify a 290 bp fragment within Region 1 of the barramundi mitochondrial control region by the polymerase chain reaction. The amplified product was sequenced using thermal cycle sequencing, interpreted with the aid of the Sequence Navigator 1.0.1 software package. Sequences from different fish were aligned by eye using MacClade 3.03 (Maddison & Maddison 1992).

Barramundi mtDNA control region sequences were compared among those localities surveyed in this study and with the wild population of the lower Ord River sampled by Doupé *et al.* (1999).

Results

This study identified a total of 19 barramundi mtDNA control region sequences, with each being 231 nucleotide bases in length. Two distinct mtDNA haplotypes were found (haplotypes A & B) and are shown in Table 1. Both haplotypes were detected at each locality; 5 A and 2 B haplotypes at the fish farm, 2 A and 6 B haplotypes in Lake Argyle, and 3 A and 1 B haplotypes in Spillway Creek. Neither the A nor the B haplotype found in this study was detected in the natural Ord River population sampled by Doupé *et al.* (1999).

Discussion

The Western Australian Department of Environmental Protection and Fisheries Western Australia are signatories to a Memorandum of Understanding concerning the translocation of aquatic species into Western Australia and between watersheds within Western Australia. Barramundi display significant population genetic structure eastwards from the Ord River in Western Australia (Shaklee *et al.* 1993; Keenan 1994; Chenoweth *et al.* 1998), and Doupé *et al.* (1999) showed substantial genetic differentiation between barramundi from the Kimberley (Ord and Fitzroy Rivers), Darwin and Cairns (Queensland). Since the depiction of Kimberley barramundi as a genetically differentiated stock, the proprietors at Lake Argyle have been encouraged to collect broodstock from the Ord River region, but the program is at a pilot stage and the Darwin hatchery remains the source of broodstock for the Lake Argyle fish farm.

Are barramundi escaping?

The identical mtDNA haplotypes found among barramundi of the fish farm, Lake Argyle and Spillway Creek provide strong evidence that barramundi have escaped from the farm at Lake Argyle. An alternative explanation is that they have migrated to Spillway Creek and Lake Argyle from the Ord River. Haplotypes A and B found in this study were not detected in the Ord River population sampled by Doupé *et al.* (1999). It is possible that haplotypes A and B are present in the natural Ord River population, but were missed by the sample of Doupé *et al.* (1999). Nevertheless, it seems a reasonable assumption that the haplotypes would occur no more frequently than $^1/_{14}$ or 0.07. If this is so, then the probability that the 8 fish sampled from Lake Argyle originated from the Ord River is $\leq 5.76\ 10^{-10}$ (0.07^8), and the probability that the 4 fish sampled from Spillway Creek originated from the Ord River is $\leq 2.40\ 10^{-5}$ (0.07^4) Clearly, these are highly unlikely events. The most parsimonious explanation, then, is that the fish in Lake Argyle and Spillway Creek escaped from the fish farm.

Hybridization between escaped and wild barramundi

The presence of escaped farmed fish in Spillway Creek suggests that they can potentially migrate from Lake Argyle to Lake Kununurra. A trial stocking of 124 tagged barramundi in Lake Kununurra recaptured fish from below the diversion dam (n = 9), and as far downstream as the Ord River estuary (C Bird, Fisheries Western Australia, pers comm). This indicates that barramundi can move from Lake Kununurra into the lower Ord River, and suggests that escaped cultured fish have the potential to genetically contribute to the natural Ord River population. The risk of hybridization then depends upon the rate of escape of cultured fish, how many survive to reproductive maturity, and then how many achieve reproductive success. The long-term effects of hybridization depend upon the effective population size (N_e) of the existing Ord River population, compared to the size of the cultured population that survives and contributes genetic material to it. N_e is extremely difficult to measure, but is usually very much smaller than the actual population size (Waples 1990). About 300-500 barramundi have been caught in Lake Argyle as a bycatch of the professional catfishery (C Ostle, Fisheries Western Australia, pers comm), and about 12-15 individual fish have been seen in Spillway Creek (Doupé, unpublished observations). How many have escaped beyond there into Lake Kununurra is not known.

The effect of hybridization on local adaptation

There is concern about genetic interaction between wild stocks and fish bred for aquaculture (Ryman 1991),

Table 1

Barramundi mtDNA control region sequences showing haplotypes A and B. A matching nucleotide base is indicated by "-". AL - Lake Argyle; SC - Spillway Creek; FF - Fish Farm.

Haplotype	Sample	Sequence
A	AL2	TCAACATTTGCTTGTAATCAAAGGACATACGTGCATTCAATGGTACTCGTAAATGCAATGTACGGTAACCTATAATTAATGTACTTCAAGCAATAATATTACATACTGATCATCAGCAATAATATGAGCGTAGTGAGAGATCACCAATCAGTAGGTATTCAGAGTGTTGACGGTTCTTGATAGTCAAGGACAGATACGGTGTGGGGGTTACACAAATTGAACTATTACTGG
	AL6	....
	SC1	....
	SC3	....
	SC4	....
	FF1	....
	FF3	....
	FF4	....
	FF6	....
	FF7	....
B	AL1	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	AL3	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	AL4	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	AL5	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	AL7	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	AL8	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	SC2	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	FF2	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....
	FF5	.... C .... T .... C .... AT .... AC .... C .... CT .... A .... A .... C ...... A .... A .... AA ....

including barramundi (Keenan & Salini 1990; Shaklee *et al.* 1993; Doupé 1997). There is a perceived risk that translocation of hatchery-reared fish could result in the establishment of less fit haplotypes, thus reducing stock fitness through the disruption of coadapted genomes (Keenan 1994). There is no clear relationship between the extent of genetic differences among populations and the degree of local adaptation; the issue is largely one of whether genetic differentiation has occurred through genetic drift or through selection (Johnson 2000).

Keenan (1994) thought that population genetic differentiation in barramundi is due to genetic drift as a result of small effective population sizes. There is, however, wide variability in life history traits among barramundi populations. For example, wild barramundi display geographic variation among localities for sex change (Moore 1979; Davis 1982), the presence of primary females (Moore 1979; Maneewong 1987), variable length/sex ratio relationships (Patnaik & Jena 1976; Moore 1979; Davis 1982) and the possibility of fully marine life histories (Pender & Griffin 1996). Such flexible life histories may be due to non-adaptive genetic differences or phenotypic plasticity, or they may be real adaptive differences. Much of the ecological diversity found among geographically disparate barramundi populations coincides with the population genetic differentiation found in the summary studies of Shaklee *et al.* (1993) and Keenan (1994). As yet, there is little ecological data for Kimberley barramundi (Doupé *et al.* 1999).

Stock management

Although demographic and ecological information is required if the influence of local selection pressures and population genetic differentiation are to be clarified, it has been argued that sustainable barramundi aquaculture in the Kimberley region of Western Australia should look toward the propogation of local stocks (Doupé 1997). However, the use of local stocks for culture will not necessarily safeguard the genetic integrity of the wild population. Aquaculturalists restrict the captive gene pool, either unintentionally through limited broodstock usage or by selecting for favoured production traits, like growth rate and disease resistance. The result of this artificial selection will be a captive population that is genetically divergent to its wild counterparts, irrespective of its origin.

The simplest and most pragmatic management approach would be to ensure that cultured fish do not escape. This could be achieved by requiring farms to provide better security, apart from predator nets, and by installing a mesh or netting mechanism at the Spillway Creek outlet that inhibited barramundi movement. A barramundi barrier at Spillway Creek would ideally be sized so as not restrict the movement of other aquatic species, such as black bream (*Hephaestus jenkinsi*), which are a significant component of the recreational fishery in Lake Kununurra, and the catfishes that support a small commercial fishery in its upper reaches. Further consideration should also be given to the expansion of commercial catfishing licences in Lake Argyle to allow the sale of barramundi caught as a bycatch; presently catfishermen are required to return all non-target species to the water.

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